



Short communication

Increase in proto-oncogene mRNA transcript levels in bovine lymphoid cells infected with a cytopathic type 2 bovine viral diarrhea virus[☆]John D. Neill^{*}, Julia F. Ridpath

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ABSTRACT

Infection of susceptible animals with bovine viral diarrhea viruses (BVDV) can result in an array of disease symptoms that are dependent in part on the strain of infecting virus and the physiological status of the host. BVDV are lymphotropic and exist as two biotypes. Cytopathic BVDV kill cells outright while noncytopathic strains can readily establish persistent infections. The molecular mechanisms behind these different affects are unknown. To gain a better understanding of the mechanisms of disease, serial analysis of gene expression (SAGE), a powerful method for global gene expression analysis, was employed to examine gene expression changes in BVDV-infected BL3 cells, a bovine B-cell lymphosarcoma cell line. SAGE libraries were constructed from mRNA derived from BL3 cells that were noninfected or infected with the cytopathic BVDV2 strain 296c. Annotation of the SAGE data showed the expression of many genes that are characteristic of B cells and integral to their function. Comparison of the SAGE databases also revealed a number of genes that were differentially expressed. Of particular interest was the increased numbers of transcripts encoding proto-oncogenes (*c-fos*, *c-jun*, *junB*, *junD*) in 296c-infected cells, all of which are constituents of the AP-1 transcriptional activation complex. Real-time RT-PCR confirmed these results and indicated that the actual increases were larger than that predicted by SAGE. In contrast, there was no corresponding increase in protein levels, but instead a significant decrease of *c-jun* and *junB* protein levels in the infected BL3 cells was observed. Rather than an increase in transcription of these genes, it appeared that these proto-oncogenes transcripts accumulated in the BVDV2-infected cells.

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Bovine viral diarrhea viruses (BVDV), members of the *Pestivirus* genus of the *Flaviviridae*, are the most economically important viral pathogens of cattle in the U.S. These viruses are present in two currently recognized biotypes, cytopathic and noncytopathic, depending on activity in epithelial cell culture. A third biotype, the lymphocytopathic biotype, has been proposed (Ridpath et al., 2006a). The noncytopathic and cytopathic biotypes differ from each other in the cleavage of the nonstructural protein NS2/3 to NS2 and NS3 proteins by the cytopathic viruses. This cleavage is mediated by a wide array of alterations in NS2/3, such as insertions of cellular mRNA sequences, duplication of viral sequences and simple amino acid substitutions (Baroth et al., 2000; Becher et al., 2002; Kummerer et al., 2000; Qi et al., 1992; Ridpath and Bolin, 1995).

Cells infected with a cytopathic BVDV generally show the classical signs of apoptotic cell death. The manner by which this protein cleavage results in cell death is currently unknown. Several reports have demonstrated that the cytopathic strains induce apoptosis. This has been shown by cleavage of genomic DNA to nucleosomal lengths (Hoff and Donis, 1997; Zhang et al., 1996), activation of caspases (Bendfeldt et al., 2003; Grummer et al., 2002; Hoff and Donis, 1997), and loss of mitochondrial membrane potential with release of Cytochrome c into the cytoplasm of the infected cell (Bendfeldt et al., 2003). The dsRNA inducible pathway has recently been implicated in killing of cells infected with cytopathic strains. These viruses produce more viral RNA, hence more dsRNA replicative intermediate, triggering the dsRNA response (Yamane et al., 2006).

BVDV causes disease of the digestive, respiratory, immune, endocrine and reproductive systems of susceptible animals. The organ systems affected and the severity of disease are strain dependent. Additionally, infection of a pregnant cow between 40 and 150 days of pregnancy can result in the birth of a persistently infected calf that is immunotolerant to the infecting strain of BVDV. The mechanism(s) that result in disease symptoms following infection with BVDV are unknown.

[☆] Product names are necessarily included to report factually on available data; however, the USDA neither guarantees nor warrants standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Serial analysis of gene expression (SAGE) was used to examine gene expression changes following infection with BVDV2. SAGE is an unbiased, quantitative approach for obtaining data regarding cellular gene expression patterns (Velculescu et al., 1995). SAGE technology generates short sequence tags (14 base tags) derived from 3' end of mRNAs in the total cellular RNA pool that are then concatamerized, cloned and sequenced. SAGE has been used previously to demonstrate gene expression changes in noncytopathic BVDV2-infected Madin–Darby bovine kidney cells (Neill and Ridpath, 2003a; Neill and Ridpath, 2003b). To gain a greater understanding of the underlying mechanisms of disease caused by BVDV, an *in vitro* model of lymphocyte infection was used to gather information from a more biologically relevant cell type than the classical epithelial cells that are commonly used to propagate BVDV. BVDV infection routinely causes a transient lymphopenia, in some cases this can be severe, and an *in vitro* lymphocyte model was seen as relevant to examining the mechanism behind this phenomenon. BL3 cells, a non-adherent bovine B-cell sarcoma cell line, were verified BVDV-free and bovine lentivirus-free by PCR (Dr. Randy Sacco, personal communication). Cells were maintained in Leibovitz MEM with Earles salts medium containing 10% BVDV- and BVDV antibody-free fetal bovine serum and were incubated at 37 °C in a 5% CO₂ atmosphere. Cells were infected with BVDV2 cytopathic strain 296c at a multiplicity of infection of 2 by addition of virus stock to the cells and incubation at 37 °C for 1 h with rocking. The medium was then replaced with fresh medium and the cells were incubated at 37 °C in a 5% CO₂ atmosphere until collected for RNA purification. Infection of BL3 cells with BVDV2 296c resulted in infection of ≥95% of the cells at 24 h post-infection as determined by immunohistochemical staining of the cells (data not shown). BL3 cells that were infected with 296c showed little morphological change at 18 h post-infection.

Total cellular RNA was purified from noninfected and BVDV2-infected cells at 18 h post-infection using Trizol reagent as recommended by the manufacturer (Invitrogen, Inc., CA). The RNA was precipitated, washed once with 75% ethanol and resuspended in 100 µl of RNase-free water. The RNA was stored at –80 °C until use in SAGE library construction or as template for PCR. RNA used for real-time PCR was DNase treated using the Turbo DNA-free kit (Ambion, Inc., Austin, TX). Quality of the RNA was determined using a 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA) according to the manufacturers instructions.

SAGE was conducted to examine changes in transcript levels following infection with 296c. Total poly(A)⁺ RNA was isolated from each RNA population and was used to construct the SAGE libraries (St Croix et al., 2000; Velculescu et al., 1995). A detailed protocol for construction of SAGE libraries can be obtained at www.sagenet.org. DNA sequencing, tag identification and data analysis were done as previously described (Neill et al., 2006). The tag sequences were extracted from the raw sequence data using SAGE 2000 ver. 4.0 software. For annotation purposes, the tag databases were merged with a database of tag-to-gene identifications prepared from the TIGR *Bos taurus* gene index (BtGI) database, release 11 (www.tigr.org/tdb/tgi/btgi/). The noninfected and 296c-infected BL3 cell SAGE databases have been submitted to GenBank and have the accession numbers GSM154615 and GSM154617, respectively. Libraries were sequenced to 74,030 and 93,538 tags, respectively. The databases were normalized to 100,000 tags with the SAGE 2000 software. The data provided by the noninfected BL3 cell library presented the normal level of transcription of genes in actively growing BL3 cells. The 25 most highly expressed proteins in BL3 cells were primarily proteins involved in protein synthesis, particularly ribosomal proteins (data not shown). Other genes that were highly transcribed included translationally controlled tumor protein, immunoglobulin lambda light chain, and guanine

Table 1

Identification and expression level of cellular transcripts in BL3 cells encoding proteins important for B-cell function

SAGE tag	Gene description	Tags	
		Noninfected	296c
Immunoglobulin			
GAGCCCGCAG	IgM heavy chain membrane form	112	89
GACCCCTGAG	Immunoglobulin lambda light chain	23	15
AGTGCAGACT	IgM heavy chain secretory form	23	10
B-cell receptor complex			
GCCACTTAGT	B-cell receptor α (Ig-α)	8	7
TCCGGCTCTC	B-cell receptor β (Ig-β)	0	1
GGGAGGAGGG	CD19	11	15
B-cell receptor positive effectors			
CCTGTGCAGT	Blk	0	1
CGGATTCCCC	Lyn	0	1
TGTGGCCTGT	Lck	0	2
B-cell receptor negative effectors			
CCTCAGCCCG	SHP1	1	5
AGGACCCCGC	Csk	1	2
Intracellular signaling proteins			
ATAGCTGGGG	Mitogen activated protein kinase kinase	1	1
GCCTCAGGAT	MAPKKK II	1	1
CGAATCAAGT	JNK kinase 2	0	2
Adaptor/linker proteins			
CAGCCTTGCA	Grb2	3	4
CATTTCCTCC	Vav1	3	0
Cell surface receptors and ligands			
ATAAACCTAA	CXC chemokine receptor 4 (CXCR4)	22	1
CTGCTGTAAT	Bovine leukemia virus receptor	2	3
CATTGGAGAA	IL 1β	4	1
TGGCCAGCCC	Semaphorin 4B	1	10
Apoptosis			
GAGGCACTGG	BAD	0	5
CGGCCCTGT	Bcl-2	0	3
CTTCTTGCC	Bcl-X _L	7	9
CCAGCCCTGT	FADD	0	4
ACTGAGGAAG	DAXX	4	6
TAGGCAAATT	Apoptosis inducing factor (AIF)	2	1
TGCAAGGACA	A1	0	1
Proto-oncogenes			
TGGAGCCAGG	<i>c-fos</i>	0	2
CGAATCAAGT	<i>c-jun</i>	1	6
GGGCGAGGGG	<i>junB</i>	0	29
ACCTCCCTG	<i>junD</i>	1	13
AACAACCTCA	<i>c-myc</i>	1	2

nucleotide-binding protein beta subunit. These transcripts did not vary significantly between the noninfected and BVDV-infected libraries.

A number of tags identified were derived from transcripts encoding proteins important in B-cell specific functions (Table 1). For the most part, these were found at similar levels in the two libraries. Exceptions were seen in the apoptotic transcripts where the numbers of some of the transcripts (Bad, bcl-2 and FADD) appear to be higher in the 296c-infected cells. The CXC chemokine receptor 4 showed a 22-fold decline in 296c-infected cells. The presence of many of these transcripts in BL3 cells was confirmed by reverse transcriptase-PCR (data not shown).

Table 2
Real-time PCR primer sets

Transcript	PCR primers ^a	Amplicon (bp)
β -actin	CGCCATGGATGATGATATTGC AAGCCGGCTTGCACAT	84
β 2-microglobulin	AGATTGCTGCACCTGTGAGATCCT TTTCTCCACTAGGCCTTCCCTCT	78
GAPDH ^b	GCATCGTGAGGGGACTTATGA GGGCCATCCACAGTCTTCTG	66
<i>c-jun</i>	AAACCTTGAAAGCGCAGAACTCGG TTGCAACTGCTGCGTTAGCATGAG	120
<i>c-fos</i>	TGAGTTGGCGCATTACAGAGAGGA CCTGGTGTGTTTACGCACAGATA	94
<i>c-myc</i>	TTTGCTCTTCGTGACCAGATCCCA TCGCCTCTTCTGCAACACGTGTAT	140
eIF-2 α	ACAACCACCCTGGAGAGAACAGAA ATCTGTGACCACCTTTGGGCTCCAT	126
eIF-4e	CCCTACTTGATAACATTAGTGATTCTG GTCATATTCTTGATCTTCACCA	99
<i>junB</i>	AGACCAGAGCGCATCAAAGTAGA TGAGTGTCTTCACTTGTCTCCA	107
<i>junD</i>	GAACAGAGTGTTCGATTCTGCCCT CCAACACAGGAGAACACACACACA	84
p53	TGAGTGCACCACTCCACTACAA AAACACGCACCTCAAAGCTGTTC	142
sec61 α	TTGTTCGGCATGACCATCACCATC CAGAAACAATCAGGCCAGCCACAAA	137
TRAM ^c	GTTGCCATGCTGCTAGAAAGTGCT AATGGCAGAGATTGTTCCACGG	113

^a top sequence plus sense; bottom sequence minus sense.

^b GAPDH – glyceraldehyde 3-phosphate dehydrogenase.

^c TRAM – translocating chain-associating membrane protein.

A detailed comparison of the SAGE databases revealed a number of additional mRNA transcripts with altered expression levels. However, the primary focus of the examination for changes in transcript numbers was to identify alterations that may be relevant to the generation of disease symptoms observed in BVDV2-infected animals. Several transcripts with numbers that showed a difference between noninfected and infected cells fit these categories. Of particular interest was the increase in the copy number of transcripts encoding several proto-oncogenes, *c-fos*, *c-jun*, *junB* and *junD* (Table 1), all constituents of the AP-1 transcriptional activation complex. Analysis of the 2 databases revealed increases of transcript numbers ranging from greater than 2-fold for *c-fos* to greater than 29-fold for *junB*. The *c-myc* mRNA showed an increase of 2-fold.

Real-time PCR was conducted to examine the levels of specific cellular mRNA transcripts following infection with BVDV using the SYBR Platinum Taq qRT-PCR kit (Invitrogen, Inc., Carlsbad, CA) with an Opticon 2 real-time PCR thermocycler (Bio-Rad, Inc., Hercules, CA). The primers that were used in this analysis are illustrated in Table 2. The data was normalized as previously described (Neill et al., 2006). Determination of the appropriate internal housekeeping control transcript for cells infected with BVDV2 strains was done using primer sets for β -actin, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and β 2-microglobulin (Biederman et al., 2004; Dheda et al., 2004; Li et al., 2005; Schmittgen and Zakrajsek, 2000). Real-time PCR for the 18S ribosomal RNA was done with the Quantum RNA 18S Internal Standard kit (Ambion, Inc.) with a PCR primer:competimer ratio of 3:7. To prepare RNA template, BL3 cells were infected with BVDV2 and the cells were collected at 3, 6, 16, and 20 h post-infection. Complete testing of these transcripts at multiple times following infection revealed that β 2-microglobulin,

β -actin and GAPDH transcript numbers declined significantly in 296c-infected cells late in infection (Fig. 1). 18S RNA was the most stable over all the times examined and infection with BVDV2 296c did not affect these transcript levels. Thus, 18S RNA was initially used to normalize real-time PCR results of the other transcripts tested (Table 3). Use of either normalized or non-normalized total RNA had no effect on the outcome of the real-time PCR results (data not shown).

The expression of multiple genes was examined using the real-time PCR. Fig. 2 illustrates the non-normalized real-time PCR data for the proto-oncogene transcripts. With the exception of *c-fos*, the levels of mRNA were essentially the same in noninfected and 296c-infected cells until at least 6 h post-infection. *c-fos* dropped roughly 2-fold following infection and did not recover until after 6 h of infection. *junD* remained at the same level as in noninfected cells until at least 16 h of infection. Most proto-oncogene mRNA levels increased at least 8-fold at 16 and 20 h post-infection.

Following normalization of the real-time PCR data, all mRNAs analyzed showed significant declines in 296c-infected cells, with the notable exception of those transcripts encoding proto-

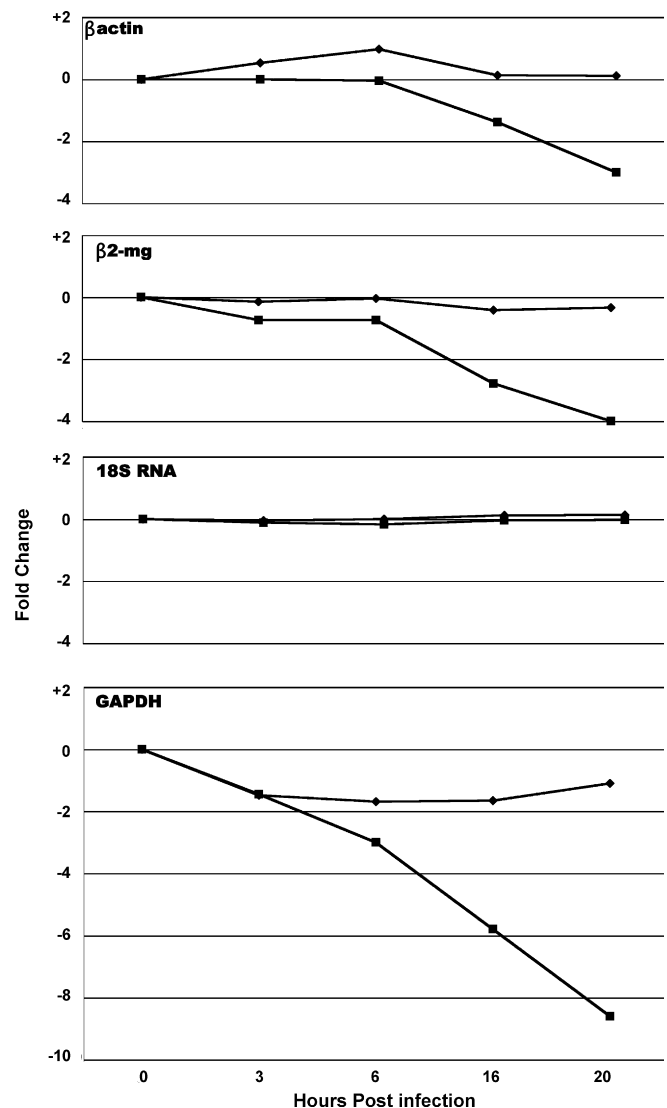


Fig. 1. Real-time PCR analysis of housekeeping genes for use in normalization of real-time PCR data. The profile of each transcript is illustrated. The diamonds represent noninfected cellular RNA and the squares represent BVDV2 296c-infected cellular RNA. This data is not normalized. β 2-mg; β 2-microglobulin.

Table 3
Real-time PCR measurement of changes in transcript numbers

Transcript	Δ RT-PCR noninfected ^a	Δ RT-PCR 296c ^a	RT-PCR β 2-mg ^b	RT-PCR 18S RNA ^c
β -actin	-2.1	-3.7	-1.1	-3.4
GAPDH	2	-2.4	-1.65	-5.3
β 2-mg	-1.4	-2.9	1	-3.2
18S RNA	1	1	3.2	1
eIF-2 α	1.3	-3.1	-1.7	-5.5
eIF-4e	1	-3.3	-1.55	-5
p53	-1.5	-5.3	-1.78	-5.9
sec61	1	-3.3	-1.23	-4
TRAM	1	-5.6	-2.42	-7.9
<i>c-fos</i>	-2.3	10.4	38.1	11.9
<i>c-jun</i>	-2.5	17.7	122.7	38.1
<i>c-myc</i>	-1.2	-4.1	-2.1	-6.7
<i>junB</i>	-1.4	8.5	47.1	14.6
<i>junD</i>	-1.73	1.8	11.1	3.4

^a Fold change in mRNA concentration between 8 and 20 h (not normalized) in BL3 cells.

^b Fold change in mRNA concentration in infected BL3 cells using β 2-microglobulin for normalization at 20 h post-infection.

^c Change in mRNA concentration in infected BL3 cells using 18S RNA for normalization at 20 h post-infection.

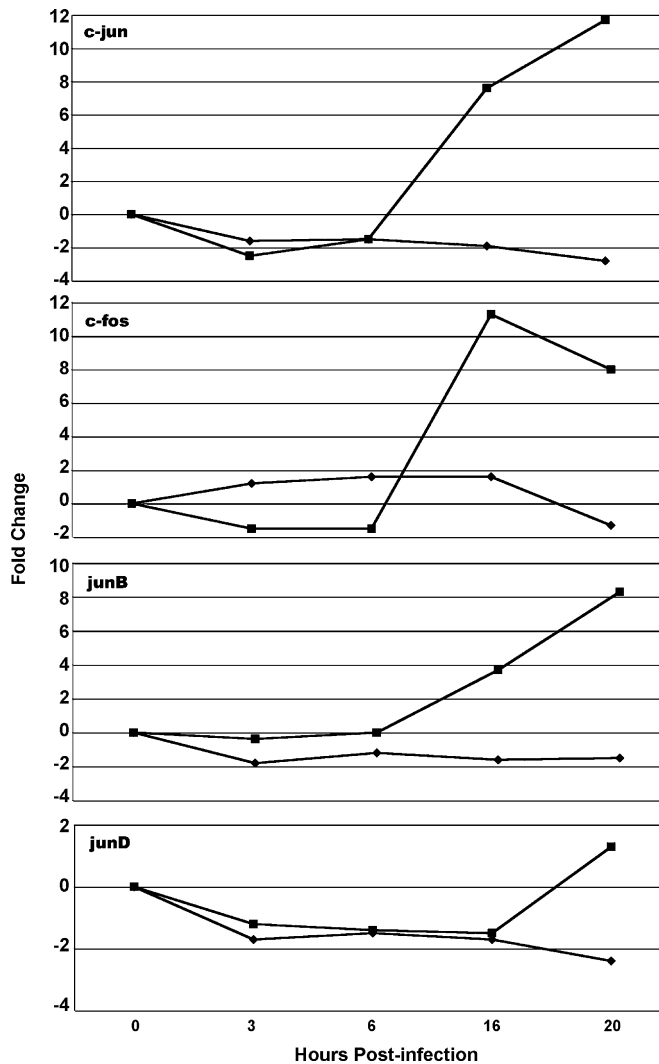


Fig. 2. Real-time PCR analysis of proto-oncogene transcripts. The profile of each transcript is illustrated. The diamonds represent noninfected cellular RNA and the squares represent BVDV2 296c-infected cellular RNA. This data is not normalized.

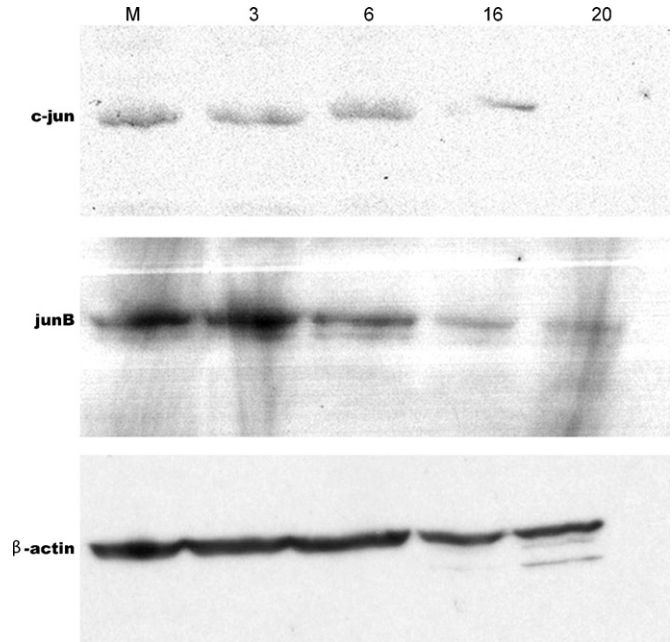


Fig. 3. Western blot analysis of *c-jun* and *junB* in mock-infected and BVDV2 296c-infected BL3 cells. Cells were collected at the times post-infection indicated above the panels. β -actin was included as a loading control. M, mock infected; collected at 20 h post-infection.

oncogenes of the AP-1 complex (Table 3). Declines in transcript numbers ranged from 3.2-fold for β 2-microglobulin to 7.9-fold for TRAM. The AP-1 proto-oncogene transcripts showed increases of 3.4-fold for *junD* to 122.7-fold for *c-jun*. Additionally, all real-time PCR results were normalized to β 2-microglobulin to illustrate proto-oncogene transcript number increases relative to a representative mRNA transcript that declined late in infection. This normalization resulted in even greater increases in the proto-oncogene transcript numbers and smaller decreases in the other mRNAs analyzed (Table 3).

Total protein lysates were prepared from noninfected and BVDV-infected BL3 cells by pelleting the cells, washing once in PBS (pH 7.2) and rapid lysis of the cells by addition of protein loading buffer (62.5 mM Tris-HCl, pH 6.9, 2% SDS, 1% glycerol and 125 mM 2-mercaptoethanol). The lysate was immediately boiled for 5 min and cooled on ice. The lysates were stored at -20°C until use. Antibodies cross-reactive to bovine β -actin (sc-1616), *c-jun* (sc-44), and *junB* (sc-73) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Western blot analysis was done to determine the amount of specific proteins present in 296c-infected cells. With the large increases observed in oncogene-encoding transcripts present in infected cells, an increase in protein levels was expected. When western blots containing total cellular proteins from noninfected and 296c-infected BL3 cells were probed with antibodies against *c-jun* and *junB*, it was readily apparent that the amounts of these protein declined in 296c-infected cells (Fig. 3). There was no observable decrease in noninfected cells. In the case of *c-jun*, the decline in protein was readily evident at 16 h post-infection and there was no detectable protein by 20 h post-infection. *junB* protein showed a decline at 6 h post-infection and a very low level at 20 h post-infection. An immunoblot using β -actin antibody was included as a loading control. The level of β -actin was equivalent between the noninfected control and the 296c-infected cells until 16 h post-infection when the amount of β -actin declined. This was the time that β -actin mRNA showed a decline of almost 2-fold (Fig. 1). At 20 h it was possible to see degradation products, probably due to

the cell death occurring in the culture. Attempts were made to find antibodies cross-reactive to bovine *c-fos* and *junD* but none were found. The results presented here can only address the levels of the *junB* and *c-jun* but it is believed at this point, that *c-fos* and *junD* protein levels are declining in a similar manner.

BVDV has long been an enigma, causing a broad spectrum of disease symptoms that involves multiple organ systems. Severity of disease is strain-dependent and the determinants are unknown. Two of the most commonly encountered effects of BVDV infection are immunosuppression and viral persistence. Both are important from a standpoint of disease control. However, the mechanisms that are involved that result in these conditions are unknown. To begin to understand these effects, a powerful gene analysis technology, SAGE, was used to obtain a global overview of gene expression in BVDV-infected cells and how it compared to gene expression levels in noninfected cells. The BL3 B-cell lymphosarcoma cell line was used as an *in vitro* bovine lymphocyte model. These cells are readily infected by BVDV and most cells are killed by cytopathic strains within 24 h of infection (Ridpath et al., 2006a). Cell death is caused by the induction of apoptosis as previously described for cultured epithelial bovine cells infected with a cytopathic strain of BVDV (Grummer et al., 2002; Ridpath et al., 2006b; Zhang et al., 1996). The effects of infection are observable and complete in a short period of time. The SAGE libraries were constructed from RNA from mock-infected and BVDV2 296c infected cells at 18 h post-infection. This is a time point before massive cell death but after the infection was well advanced.

Real-time PCR was used to examine the relative levels of selected transcripts to corroborate SAGE results. To determine the best housekeeping gene internal control, 3 mRNAs and a ribosomal transcript were tested at increasing times post-infection. Of the 3, only the ribosomal 18S RNA transcript remained unchanged over time in 296c-infected cells. The mRNA transcripts, β -actin, β 2-microglobulin and GAPDH, showed significant declines in numbers as the infection progressed (Fig. 1) as did most of the other transcripts examined (Table 3). Because of this, the real-time PCR data was normalized with both 18S RNA and β 2-microglobulin to define fold change in proto-oncogene and other cellular transcripts. It was believed that normalization with β 2-microglobulin was a more accurate indicator of the actual state of mRNA synthesis/turnover in the 296c-infected cells. Analysis with real-time PCR showed that the *c-fos*, *c-jun*, *junB* and *junD* transcripts were indeed present at elevated levels in 296c-infected cells while the remainder of the mRNA transcripts analyzed showed sharp declines in numbers at or later than 16 h of infection (Table 3; Fig. 2). Normalization with 18S RNA indicated an increase in transcript numbers of these 4 proto-oncogene transcripts and a decline in the other transcripts examined. Normalization with β 2-microglobulin resulted in larger increases in the 4 proto-oncogene transcripts and smaller decreases with the other transcripts. Significantly, the *c-jun* transcripts showed an increase of over 122-fold. The 4 proto-oncogene transcripts were present at elevated levels regardless of the normalization method. The only other proto-oncogene transcript included in this analysis was *c-myc*. This transcript showed a declining pattern late in infection similar to that observed with the non-proto-oncogene transcripts (Table 3), even though SAGE showed a modest 2-fold increase (Table 1). The cause of this discrepancy is unknown.

Western blots of *junB* and *c-jun* revealed sharp decreases in protein levels in the 296c-infected cells, occurring in the face of large increases in the proto-oncogene transcript levels of 47- and 122-fold, respectively. Increases in mRNA and declines in protein indicate that the transcripts may be translationally repressed. The mechanism behind this observation is unclear. The AP-1 transcriptional activator is important in the immediate early pro-growth

response. All of the AP-1 proto-oncogene encoding transcripts possess an AU-rich element (ARE) in their 3' UTRs that destabilize the transcripts and cause rapid turnover to limit expression of the oncogene proteins. Additionally, the BVDV genomic RNA contains an ARE near the 3' end. Translational repression was demonstrated in human papillomavirus-1 late transcripts that contained an ARE in the 3' UTR (Wiklund et al., 2002). This repression was completely dependent on the presence of the poly(A) tract and involved interaction with the poly(A)-binding protein. In a non-viral translational repression system, Wax et al. (2005) demonstrated that translation of the tumor necrosis factor- α mRNA was repressed by cellular proteins binding to the ARE in the 3' UTR. This prevented the association of the 43S ribosomal complex with the transcript and was independent of the presence of a poly(A) tract. Preliminary experiments were done where the ARE-containing sequences from the 3' UTR of the proto-oncogene transcripts and BVDV genomic RNA were placed downstream of the GFP open reading frame (data not shown). The results indicated that all of the ARE-containing sequences were highly destabilizing, but interestingly, the BVDV 3' sequences were more strongly destabilizing than the highly studied *c-fos* ARE. Thus, there must be some means to protect the BVDV genomic RNA from degradation by normal cellular degradation pathways, and possibly, this mechanism is also protecting these proto-oncogene transcripts from degradation. Further experiments are underway to examine the effect of BVDV infection on proto-oncogene transcript abundance.

SAGE revealed that there were some significant changes in gene expression in BVDV-infected cells. Some of the most profound and unexpected were increases in the transcripts encoding components of the AP-1 transcriptional complex. It is unknown the reason for the apparent accumulation of these transcripts and the downstream effects of loss of expression of the oncogene products. The lack or decline of the AP-1 transcriptional activator complex may contribute to cell death in cells infected with cytopathic BVDV strains. It seems highly likely though, that if the proto-oncogene transcripts are protected in the same manner as the BVDV genomic RNA, it is only fortuitous and not a protective mechanism of the virus.

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